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# Formation of Polyion Complex Micelles in an Aqueous Milieu from a Pair of Oppositely-Charged Block Copolymers with Poly(ethylene glycol) Segments

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ABSTRACT: Stable and monodispersive polyion complex micelles were prepared in an aqueous milieu through electrostatic interaction between a pair of oppositely-charged block copolymers with poly(ethylene glycol) segments: poly(ethylene glycol)–poly(L-lysine) block copolymer (PEG–P(Lys)) and poly(ethylene glycol)–poly(α,β-aspartic acid) block copolymer (PEG–P(Asp)). It was confirmed from photon correlation spectroscopy (dynamic light scattering) that the scaled average characteristic line width ($\Gamma/K^2$) was independent of the magnitude of the scattering vector ($K^2$), and the diffusion coefficient ($D_T$) kept constant regardless of the concentration, indicating that the polyion complex micelles were spherical particles without any secondary aggregates. Further, polydispersity indexes ($\mu_2/\Gamma^2$) were always less than 0.1 in the range of the measured concentration (1–10 mg/mL). The hydrodynamic radius at infinite dilution of polyion complex micelles was then determined to be 15.2 nm by using the Stokes–Einstein equation. The unimodal size distribution with $d_w/d_n$ of 1.07 was confirmed from the correlation function profile by the histogram analysis. The size of polyion complex micelles was unchanged even after a 1-month storing, suggesting that the polyion complex micelles are in thermodynamic equilibrium. Viscosity measurement as well as laser–Doppler electrophoresis provided evidence of the stoichiometry of the polyion complex micelles formation. These polyion complex micelles have potential utility as vehicles for charged compounds, i.e., proteins and nucleic acids, in the field of drug delivery.

## Introduction

Recently, supramolecular assemblies formed through the association of macromolecules have received considerable attention in various fields.[1,2] Particularly, many intensive studies have been carried out on the association of block copolymers in a selective solvent to form polymer micelles with a core–shell structure.[3,4] Block copolymer micelles are characterized by their size (several tens of nanometers) and stability (low critical association concentration).[5–8] Further, the inner core may act as a microreservoir for various substances.[9–11] These unique characteristics offer a special advantage in using micelles of amphiphilic block copolymers in an aqueous milieu for various applications including separation technologies[12] and drug delivery systems (drug targeting).[13,14] We have recently succeeded in developing a stable micelle system of an amphiphilic block copolymer with a core charged with an anticancer drug (doxorubicin) and have verified its excellent utility for targeting therapy of solid tumors.[15–18]

The formation of a corona of hydrophilic segments surrounding the core of water-incompatible segments is the reason for prevention of progressive aggregation of the core and for stabilization of the micelles of amphiphilic block copolymers in an aqueous milieu. Obviously, this concept of polymer micelle stabilization in an aqueous milieu by the hydrophilic corona can be extended to include macromolecular association through a force other than hydrophobic interaction.

In this study, we report the preparation of stable and considerably monodispersive ($\mu_2/\Gamma^2 < 0.1$) polymer micelles through electrostatic interaction between a poly(ethylene glycol)–polycation block copolymer and a poly(ethylene glycol)–polyanion block copolymer in an aqueous milieu. We selected biodegradable poly(L-lysine) and poly(aspartic acid) as the polycation and polyanion segments in the block copolymer, respectively, with the intention of using these polymer micelles as novel drug carriers.

## Experimental Section

**Materials.** ε-(Benzyloxycarbonyl)-L-lysine and β-benzyl L-aspartate were purchased from Peptide Institute, Inc., Japan, and used without further purification. Bis(trichloromethyl) carbonate (triphosgene) was purchased from Tokyo Kasei Kogyo Co., Ltd., Japan, and used without further purification. α-Methoxy-ω-aminopoly(ethylene glycol) (PEG; $M_n$ = 5000, $M_w/M_n$ = 1.05, functionality of amino group, 0.956) was a kind gift from Nippon Oil & Fats Co., Ltd., Japan. PEG was reprecipitated into 100-fold diethyl ether from a chloroform solution (1.0 g/mL) at room temperature. Then, the precipitated polymer was dissolved in benzene (1.0 g/mL), followed by freeze-drying to obtain the sample used for the block copolymer synthesis. Trifluoroacetic acid, anisole, methanesulfonic acid, and triethylamine were purchased from Wako Pure Chemical Industries, Ltd., Japan, and used without further purification. Tetrahydrofuran (THF), *n*-hexane, *N,N*-dimethylformamide (DMF), and chloroform were doubly distilled by general methods.[19] 0.01 N HCl and 0.01 N NaOH (titration grade) were purchased from Wako Pure Chemical Industries, Ltd., Japan.

**Synthesis of Block Copolymers. *N*-Carboxyanhydride of ε-(Benzyloxycarbonyl)-L-lysine.** Synthesis of *N*-carboxyanhydride of ε-(benzyloxycarbonyl)-L-lysine (Lys(Z)–NCA) was carried out by the Fuchs–Farthing method using triphosgene.[20] A total of 24.8 g (88.6 mmol) of Lys(Z) was suspended in 225 mL of THF. Then 11.6 g (39.1 mmol) of triphosgene was dissolved in 25 mL of THF and added to the suspension of Lys(Z). The reaction mixture was heated to 50 °C under stirring. After the reaction mixture became transparent (approximately 30 min), the solvent was evaporated under reduced pressure. The obtained Lys(Z)–NCA was recrystallized three times from a mixture of THF/*n*-hexane and dried at room temperature *in vacuo*. The yield was 24.8 g (91.5%). The purity was checked by the melting point which was determined to be 99.2–101.8 °C, corresponding to the known melting point of Lys(Z)–NCA (101 °C) prepared by a general

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Fuchs–Farthing method.[21] The narrow range of the melting point indicated that the obtained Lys(Z)–NCA was of adequate purity.

**Poly(ethylene glycol)–Poly[ε-(benzyloxycarbonyl)-L-lysine] Block Copolymer (PEG–P(Lys(Z))).** A total of 4.90 g (16.0 mmol) of Lys(Z)–NCA was dissolved in 50.0 mL of DMF. Then 4.01 g (0.80 mmol) of α-methoxy-ω-aminopoly(ethylene glycol) was dissolved in 60.0 mL of DMF and added to the solution of Lys(Z)–NCA. The reaction mixture was stirred for 20 h at 40 °C under a dry argon atmosphere. The polymerization was stopped when the peaks corresponding to *N*-carboxyanhydride peaks (1850, 1760, and 915 $cm^{-1}$) disappeared from the IR spectrum. DMF was then evaporated under reduced pressure. The resulting product was dissolved in 90.0 mL of chloroform and then precipitated into 1.0 L of diethyl ether. The yield was 6.79 g (82.7%). The composition of PEG–P(Lys(Z)) was determined by 400-MHz $^1$H-NMR (JEOL EX400) in DMSO-$d_6$. GPC measurement on the block copolymer was carried out at 40 °C in THF using HLC-8020 (Tosoh) equipped with a column combination of TSK-Gel G4000HxL, G3000HxL, and G2000HxL columns. The signals were detected by a UV detector at 254 nm.

**Poly(ethylene glycol)–Poly(L-lysine) Block Copolymer (PEG–P(Lys)).** A total of 1.30 g of PEG–P(Lys(Z)) was dissolved in 13.0 mL of trifluoroacetic acid and stirred for 1 h. Then, the solution was added to 13.0 mL of anisole and 9.0 mL of methanesulfonic acid and stirred for 1.5 h. Subsequently, the solution was diluted with 65.0 mL of distilled water and was vigorously shaken with 300 mL of diethyl ether in order to remove the anisole and the acids in excess. This process was repeated until the diethyl ether phase became neutral. The water phase was neutralized by triethylamine and dialyzed against distilled water using a Spectrapor 6 dialysis membrane (molecular weight cutoff = 1000). The PEG–P(Lys) was obtained as a white powder after lyophilization. The yield was 0.93 g (81.3%). $^1$H-NMR measurement (JEOL EX400) of the obtained PEG–P(Lys) was carried out in $D_2O$ to determine the composition.

**Poly(ethylene glycol)–Poly(aspartic acid) Block Copolymer (PEG–P(Asp)).** PEG–P(Asp) was synthesized according to the procedure previously reported.[22,23] Briefly, poly(ethylene glycol)–poly(β-benzyl L-aspartate) block copolymer (PEG–PBLA) was prepared by the polymerization of β-benzyl L-aspartate–*N*-carboxyanhydride (BLA–NCA) initiated by the terminal amino group of α-methoxy-ω-aminopoly(ethylene glycol). A similar procedure was used for PEG–P(Lys(Z)). A mixture of doubly distilled chloroform and DMF (volume ratio: 1/10) was used as the reaction solvent. Benzyl groups of PEG–PBLA were removed by 0.1 N NaOH to obtain PEG–P(Asp). $^1$H-NMR measurement (JEOL EX400) of the obtained PEG–P(Asp) was carried out in $D_2O$ to determine the composition as well as to analyze the α to β isomerization of aspartic acid units during debenzylation.

**Titration of Block Copolymers.** A total of 70 mg of PEG–P(Lys) was dissolved in 30 mL of 0.01 N HCl and titrated with 0.01 N NaOH using an automatic titrator (DL-25, Mettler). The titrant was added in 0.01–mL quantities at 12–120-s intervals. Similarly, titration of PEG–P(Asp) (70 mg in 30 mL of 0.01 N NaOH) was carried out using 0.01 N HCl as the titrant. The dissociation degree at a given pH was then calculated from the titration curves.

**Preparation of Polyion Complex Micelles.** Given amounts of PEG–P(Lys) and PEG–P(Asp) were dissolved in a phosphate buffer solution (PBS, 10 mM, pH 7.29; $Na_2HPO_4$, 1.420 g/L; $NaH_2PO_4{\cdot}2H_2O$, 0.997 g/L). These solutions were purified by passing through a 0.1-μm filter (MILLEX-VV, Millipore) to remove dust. Polyion complex micelles were then prepared by mixing these solutions in an equal unit ratio of L-lysine and aspartic acid residues in the block copolymers.

**Dynamic Light Scattering Measurements.**[24,25] Dynamic light scattering (DLS) measurements were carried out by using a DLS-700 instrument (Otsuka Electronics Co., Ltd.). Vertically polarized light of $\lambda_0$ = 488 nm wavelength from an Ar ion laser was used as an incident beam. All measurements were performed at 23.5 °C. All samples were purified by passing through a 0.45-μm filter (13-HV, Millipore).

The general formula for the photoelectron count time correlation function has the form

$$g^{(2)}(\tau) = 1 + \beta|g^{(1)}(\tau)|^2 = 1 + \beta \exp(-2\Gamma\tau) \quad (1)$$

where $g^{(2)}(\tau)$ is the normalized second-order correlation function, $\beta$ is a parameter of the optical system (constant), $g^{(1)}(\tau)$ is the normalized first-order correlation function, $\tau$ is the delay time, and $\Gamma$ is the average characteristic line width. In the case of polydisperse systems, $g^{(1)}(\tau)$ can be expressed by the following equation:

$$g^{(1)}(\tau) = \int G(\Gamma) \exp(-\Gamma\tau)\, d\Gamma \quad (2)$$

where $G(\Gamma)$ is a distribution function of $\Gamma$, $K$ is the magnitude of the scattering vector, and $n$ is the refractive index. In the present analysis, the autocorrelation functions were analyzed using the method of cumulants in which

$$g^{(1)}(\tau) = \exp[-\Gamma\tau + (\mu_2/2)\tau^2 - (\mu_3/3!)\tau^3 + \ldots] \quad (3)$$

yielding an average line width, $\Gamma$, and a variance (polydispersity index), $\mu_2/\Gamma^2$.

For spherical particles, $\Gamma$ is related to the translational diffusion coefficient $D_T$ provided that the internal motions are negligible. In the cumulant approach, the *z*-averaged translational diffusion coefficient was obtained from the average line width, $\Gamma$, based on the following equation

$$\Gamma = D_T K^2 = 4\pi n \sin(\theta/2)/\lambda \quad (4)$$

In the dilute concentration region, the concentration dependence of the translational diffusion coefficient $D_T$ can be expressed by a first-order expansion;

$$D_T = D_0(1 + k_d C) \quad (5)$$

where $D_0$ is the translational diffusion coefficient at infinite dilution, $k_d$ is the diffusion second virial coefficient, and $C$ is the concentration. The corresponding hydrodynamic radius $R_h$ can then be calculated by using the Stokes–Einstein equation:

$$R_h = k_B T/(6\pi\eta D_0) \quad (6)$$

where $k_B$ is the Boltzmann constant, $T$ is the absolute temperature, and $\eta$ is the solvent viscosity.

The estimation of the size distribution was carried out from the correlation function profile by the histogram analysis software. In the histogram method,[26] eq 2 is replaced by

$$g^{(1)}(\tau) = \sum G(\Gamma_i) \exp(-\Gamma\tau)\Delta\Gamma \quad (7)$$

and $G(\Gamma_i)$ was determined using the Marquart nonlinear least-squares routine. $G(\Gamma_i)$, which is the distribution according to the ratio of light scattering by the particles with $\Gamma$, was then converted into the particle size distribution $G(d)$, using eqs 4 and 6. The distribution according to the weight ratios and the number ratios was then determined from $G(d)$.

**Laser–Doppler Electrophoresis Measurement.** Laser–Doppler electrophoresis measurement was carried out by using an ELS-800 instrument (Otsuka Electronics Co., Ltd.). The measurement was performed at 15.5 °C with an electrical field strength of 32–36 V/cm. This instrument measures the particle velocity using a laser light scattering technique. Due to the Doppler effect, the frequency of the scattered laser light is different from the frequency of the original laser beam. This frequency shift, the Doppler frequency, is related to the particle velocity. The relationship between the frequency shift and the electrophoretic mobility is expressed by the following equation:

$$u = (\nu_d \lambda)/[2En \sin(\theta/2)] \quad (8)$$

where $\nu_d$ is the Doppler frequency, $u$ is the electrophoretic

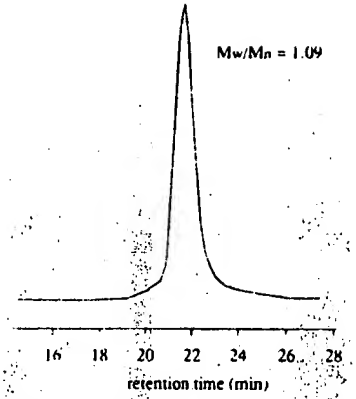


**Figure 1.** GPC chromatogram of PEG−P(Lys(Z)) (columns TSK-Gel G4000HxL, G3000HxL, and G2000HxL; eluent, THF; flow rate, 1.0 mL/min; UV detect, 254 nm; temperature, 40 °C).

mobility, $E$ is the electrical field strength, $n$ is the refractive index, $\lambda$ is the wavelength of the original laser beam, and $\theta$ is the scattered angle.

From the determined electrophoretic mobility, the zeta-potential ($\zeta$) was calculated by the Smoluchouski equation as follows:

$$\zeta = 4\pi\eta u/\epsilon \qquad (9)$$

where $\eta$ is the viscosity of the solution and $\epsilon$ is the dielectric constant of the solvent.

**Viscosity Measurements.** Viscosity measurements were carried out by using a Cannon−Fenske viscometer (Shibata Scientific Technology, Ltd.). Mixed solutions of block copolymers were prepared with varying mixing compositions of two block copolymers, keeping the total concentration constant (1.0 mg/mL). Solution flow times were measured at 30 °C. The average flow time was calculated from triplicate measurements, and the kinematic viscosity ($\eta$) was calculated by the following equation:

$$\text{kinematic viscosity} = \text{constant of viscometer} \times \text{flow time} \qquad (10)$$

The kinematic viscosity of the solvent ($\eta_0$), distilled water, was also measured, and the relative viscosity ($\eta/\eta_0$) was then calculated.

## Results and Discussion

**Synthesis and Characterization of Block Copolymers.** In this study, α-methoxy-ω-aminopoly(ethylene glycol) (PEG) was used as an initiator for NCA polymerization. The primary amine at the chain end of PEG attacks exclusively the carbonyl carbon (C-5) of the NCA ring to initiate the polymerization. As primary aliphatic amines are more nucleophilic than the active chain ends, initiation is faster than propagation.[27] Consequently, all initiator molecules (in this case, PEG) should be incorporated into the growing peptide chain to obtain a block copolymer of PEG and protected poly(amino acid) (PEG−PBLA or PEG−P(Lys(Z))). Figure 1 shows a GPC chromatogram of the obtained PEG−P(Lys(Z)). It has a narrow molecular weight distribution, and $M_w/M_n$ was determined to be 1.09. Figure 2 shows $^1$H-NMR spectra of PEG−P(Lys(Z)) in DMSO-$d_6$ as well as of PEG−P(Lys) in $D_2O$, which was prepared from PEG−P(Lys(Z)) by the deprotection of ϵ-(benzyloxycarbonyl) groups. From the peak intensity ratio of methylene protons of PEG ($OCH_2CH_2$: $\delta$ = 3.6 ppm) and phenyl protons of the ϵ-(benzyloxycarbonyl) group of P(Lys(Z)) ($CH_2C_6H_5$: $\delta$ = 7.3 ppm), the polymerization

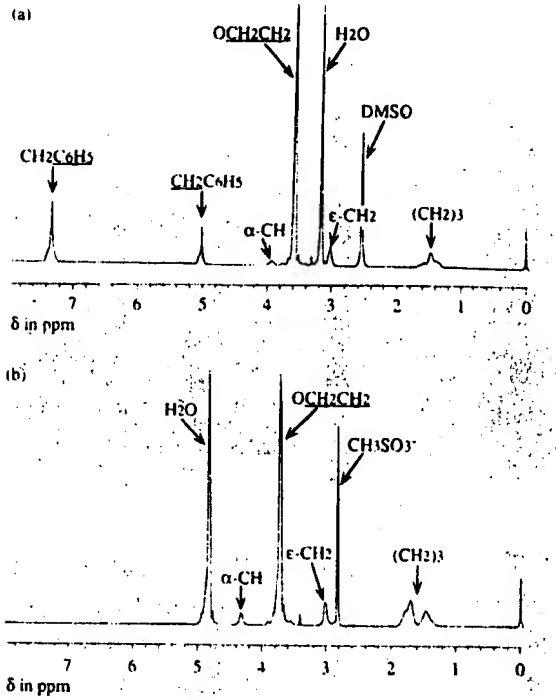


**Figure 2.** $^1$H-NMR spectra of (a) PEG−P(Lys(Z)) in DMSO-$d_6$ (temperature: 80 °C) and (b) PEG−P(Lys) in $D_2O$ (temperature: 20 °C).

degree (DP) of Lys(Z) was calculated to be 18. For PEG−P(Lys), the peak intensity ratio of methylene protons of PEG ($OCH_2CH_2$: $\delta$ = 3.7 ppm) and ϵ-methylene protons of P(Lys) ($(CH_2)_3CH_2NH_3$: $\delta$ = 3.0 ppm) was measured to calculate the DP value which was determined to be 18. Both calculations for PEG−P(Lys(Z)) and PEG−P(Lys) gave the same DP value of lysine units (DP = 18), indicating that a loss of lysine units by the main-chain cleavage during the deprotection was negligible.

As is the case of PEG−P(Lys(Z)), the unimodal distribution of the PEG−PBLA was confirmed by GPC.[28] $^1$H-NMR measurement in $CDCl_3$ was performed to determine the composition (data not shown). From the ratio of the peak intensity of methylene protons of PEG ($OCH_2CH_2$: $\delta$ = 3.6 ppm) and benzyl protons of PBLA ($COOCH_2C_6H_5$: $\delta$ = 5.0 ppm), the degree of polymerization for BLA was determined to be 19. $^1$H-NMR measurement in $D_2O$ after deprotection of the PBLA blocks was also performed, and the result is shown in Figure 3. The polymerization degree calculated from the intensity ratios of the peaks, PEG ($OCH_2CH_2$: $\delta$ = 3.7 ppm) and methylene of α-,β-amide of P(Asp) ($\delta$ = 2.7 ppm), was DP = 19 which is also in a good agreement with the DP value before the deprotection. It should be noted that the racemization as well as intramolecular isomerization of aspartic acid units to form β-aspartate units occurred in the process of the deprotection.[15,23] The ratio of α and β units in the P(Asp) segment was determined by $^1$H-NMR to be 1:3 (Figure 3). This is in good agreement with a recently reported value of the ratio of α:β linkages of sodium polyaspartate prepared from polysuccinimide under a variety of hydrolysis conditions: one- and two-dimensional NMR characterization of sodium polyaspartate revealed that α and β linkages have random sequencing in the chain of a 1:3 ratio.[29]

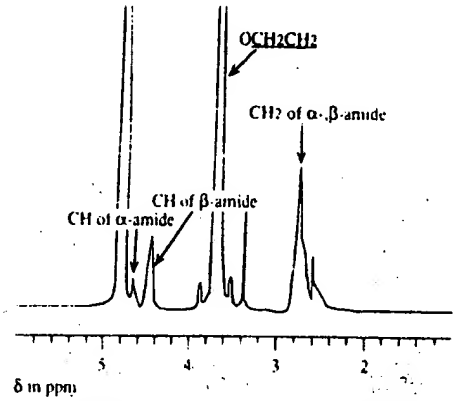


**Figure 3.** $^1$H-NMR spectrum of PEG–P(Asp) in $D_2O$ (temperature: 20 °C).

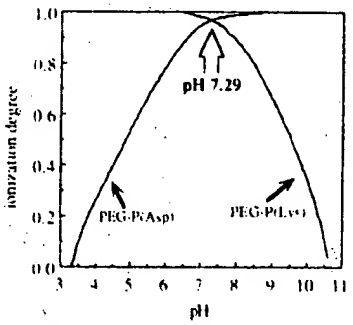


**Figure 4.** Change in the dissociation degree with pH for poly(ethylene glycol)–poly(L-lysine) and poly(ethylene glycol)–poly(aspartic acid) block copolymers (temperature: 25 °C).

Figure 4 shows the relationship between pH and the dissociation degree for PEG–P(Lys) and PEG–P(Asp), respectively, calculated from the titration results. The $pK_a$ values for PEG–P(Lys) and PEG–P(Asp) were 9.54 and 4.88, respectively. P(Lys) and P(Asp) homopolymers having similar molecular weights as the poly(amino acid) segment of the two block copolymers have $pK_a$ values of 10.03 and 4.93, respectively. Thus, the $pK_a$ value of the P(Asp) segment in the block copolymer (PEG–P(Asp)) was almost equal to the value of the homopolymer. It is interesting to note that the $pK_a$ value of the P(Lys) segment in the block copolymer (PEG–P(Lys)) was slightly lower ($\Delta pK_a \sim 0.49$) than that of the homopolymer. This difference may be attributed to the intramolecular interaction between P(Lys) and PEG segments of the block copolymer which stabilized the α-helix structure of the P(Lys) segment. The details of this intramolecular interaction will be reported elsewhere.[30]

The following experiments on polyion complex micelles were carried out at pH 7.29, where both block copolymers have the same ionization degree ($\alpha = 0.967$).

**Formation and Analysis of Polyion Complex Micelles.** Polyion complex micelles were prepared by mixing 5.0 mg/mL PBS solutions of PEG–P(Lys) and PEG–P(Asp) under the condition that the unit ratio of L-lysine and aspartic acid residues in the block copolymers were equal (electrostatically neutralized condition). The mixture was stored overnight at room temperature before DLS measurement. In order to confirm whether polyion complex micelles have a spherical structure, angle-trace DLS measurements were carried out at 30°, 45°, 60°, 90°, 120°, 135°, and 150°. Figure 5 shows the relationship between the scaled average characteristic line width ($\Gamma/K^2$) and the magnitude of the scattering vector ($K^2$). For spherical particles, the $\Gamma/K^2$ values should be independent of the detection angle because of the undetectable rotational motion. Our result, presented in Figure 5, showed that the $\Gamma/K^2$ values of the polyion complex micelle were independent of the detection angle, indicating the micelle is spherical in nature. Since the angular dependence of $\Gamma/K^2$ is negligible, the following DLS measurements were performed at 90°.

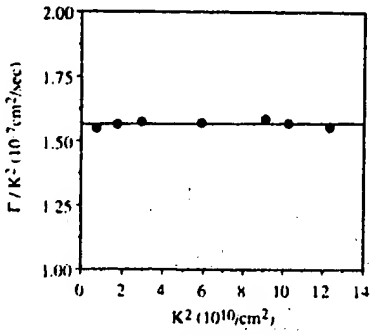


**Figure 5.** Relationship between the scaled average characteristic line width ($\Gamma/K^2$) and the magnitude of the scattering vector ($K^2$) (total concentration, 5.0 mg/mL; temperature, 23.5 °C; detection angles, 30°, 45°, 60°, 90°, 120°, 135°, and 150°).

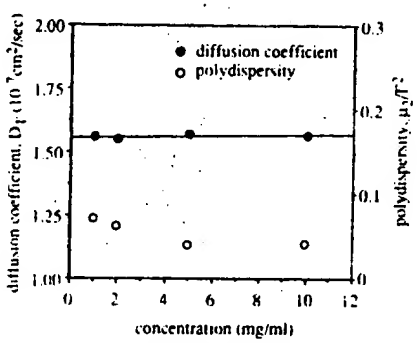


**Figure 6.** Plots of translational diffusion coefficient ($D_T$) (●) and polydispersity ($\mu_2/\Gamma^2$) (○) against the total concentration of polyion complex micelles (temperature, 23.5 °C; detection angle, 90°).

Figure 6 shows the concentration dependence of the diffusion coefficient ($D_T$) and the polydispersity ($\mu_2/\Gamma^2$). Micelles were prepared by mixing the solutions of PEG–P(Lys) and PEG–P(Asp) under electrostatically neutralized condition (1:1 unit ratio of lysine and aspartic acid residues). The total concentration of PEG–P(Lys) and PEG–P(Asp) in the mixed solutions was given by the abscissa. It is obvious that the diffusion coefficients were independent of the concentration and the diffusion second virial coefficient ($k_D$) in eq 5 was almost zero. Then, the diffusion coefficient at infinite dilution $D_0$ was determined from Figure 6 to be $1.5563 \times 10^{-7}$ cm²/s. From this value, the hydrodynamic radius $R_h$ of 15.2 nm was calculated by using the Stokes–Einstein equation (eq 6). The polydispersity ($\mu_2/\Gamma^2$) is less than 0.1 regardless of the concentration, indicating that the micelles have a considerably narrow size distribution and are essentially monodispersive. A negligible angular dependence of $\Gamma/K^2$, as shown in Figure 5, is

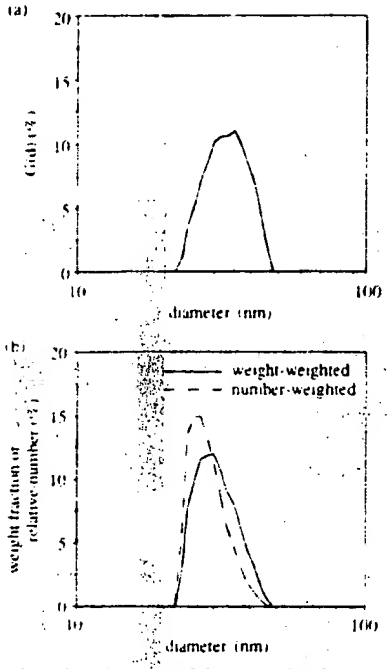


**Figure 7.** Size distributions of the $z$-weighted (a) and weight-weighted (solid line) and number-weighted (dashed line) (b) values for polyion complex micelles measured by dynamic light scattering (total concentration, 5.0 mg/mL; temperature, 23.5 °C; detection angle, 90°). Weight- and number-average diameters were determined to be 30.8 nm ($d_w$) and 28.8 nm ($d_n$), respectively, with a polydispersity ($d_w/d_n$) of 1.07.

consistent with this narrow size distribution. Further, the diffusion coefficient was unchanged even after 30 days of storing at room temperature, suggesting that the micelles are in a thermodynamic equilibrium.

Figure 7 shows the $z$-weighted (Figure 7a) and the weight- and number-weighted size distributions (Figure 7b) analyzed by the histogram method for the micelle solution in 10 mM PBS (5 mg/mL, pH 7.29). Obviously, the distribution is monodispersive with an average diameter of ca. 30 nm. This is in a good agreement with $R_h$ of 15.2 nm determined by the cumulant approach. Using the histogram method, the polydispersity ($d_w/d_n$) defined as the ratio of the weight-averaged diameter and the number-averaged diameter was also determined. The $d_w/d_n$ value based on the data shown in Figure 7b was calculated to be 1.07.

In sharp contrast with the block copolymer system, a mixture of poly(L-lysine) homopolymer and poly($\alpha,\beta$-aspartic acid) homopolymer was found to form polydispersive droplets, so-called complex coacervate (data not shown). The complex coacervate is formed through liquid–liquid phase separation and is a highly concentrated phase of two oppositely-charged weak polyelectrolytes loosely associated through electrostatic interaction.[31] The process of complex coacervation begins from the formation of small associates followed by the progressive coalescence of the associates to develop droplets and ends up in the formation of a macroscopic dual phase consisting of very diluted and concentrated polymer solutions. Coacervate droplets are metastable and polydispersive with variable diameters in the range of several tens of micrometers.

It is now well accepted that block copolymers in a selective solvent form micelles composed of a relatively

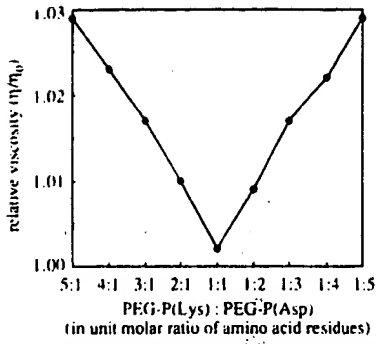


**Figure 8.** Viscosity of the PEG–P(Lys)/PEG–P(Asp) system with varying mixing molar ratios of amino acid residue (total concentration, 1.0 mg/mL; temperature, 30 °C).

compact core of the insoluble blocks and a highly swollen corona of the soluble blocks.[3-8,32,33] Presumably, in our case, the micellization of PEG–P(Lys) and PEG–P(Asp) follows a process of the association of two oppositely-charged segments to form the core of polyion complexes. Unlike homopolymer mixtures, further coalescence of micelles to form larger coacervate droplets was not observed in the block copolymer system. These results suggest that the phase mixing of PEG with polyion complexes should be thermodynamically unfavorable, assuring a stable and monodispersive distribution of the micelles in a particular size range ($R_h \approx 15$ nm). It should be noted that this size range is quite similar to that reported for the block copolymer micelles known to have a core–shell structure.[25]

**Stoichiometry of Polyion Complex Micelles.** Figure 8 shows the results of viscosity measurements of the PEG–P(Lys)/PEG–P(Asp) system with a varying mixing molar ratio. In this experiment, the total concentration of polymer (PEG–P(Lys) + PEG–P(Asp)) was kept constant ($C$ = 1.0 mg/mL). When the molar ratio of the Lys/Asp residue was unitary, the relative viscosity $\eta/\eta_0$ took a minimum value, indicating that the complexation of two block copolymers proceeded stoichiometrically. A decrease in the $\eta/\eta_0$ value is in line with the phase separation of the block copolymer to form micelle structure. Further, based on the laser–Doppler electrophoresis, the electrophoretic mobility of the polyion complex micelles was determined to be $-0.082 \pm 0.021$. The average $\zeta$-potential was then calculated from this value, using the Smoluchouski equation (eq 9). The average $\zeta$-potential of polyion complex micelles calculated had an extremely small absolute value ($-1.270 \pm 0.0320$ mV), indicating the electrical neutrality of polyion complex micelles. This result is consistent with the viscosity measurement where the stoichiometry of polyion complex micelle formation was indicated.

## Conclusion

Mixing of oppositely charged block copolymers in an aqueous milieu led to the spontaneous formation of polyion complex micelles having a spherical shape with a considerably narrow distribution. As for other block copolymer micelles, these polyion complex micelles may have a corona of hydrophilic PEG segments which surround the core of the polyion complex of cationic and anionic segments. It is well-known that various charged substances such as ions, proteins, and nucleic acids are selectively concentrated in polyion complex coacervates through electrostatic interaction.[34] This feature is quite feasible for utilizing the polyion complex micelles made

from block copolymers as vehicles for charged compounds in the field of drug delivery. Recently, in our group, preliminary results have been obtained for the inclusion of peptides into the polyion complex micelles.[35] Also, the core of the polyion complex micelles should provide a unique field for chemical reactions because it forms a segregated phase from the outer aqueous entity, and substance condensation should accelerate certain chemical reactions, as in the case of coacervate droplets.

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